174. *Experiments on the Synthesis of Anthocyanins. Part XX. Synthesis of Malvidin 3-Galactoside and its Probable Occurrence as a Natural Anthocyanin.*

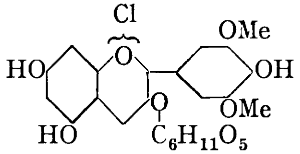
By (Miss) JANET C. BELL and ROBERT ROBINSON.

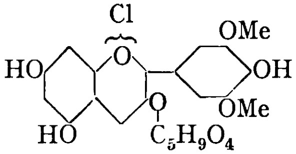
ALTHOUGH the anthocyanins of the fruits of *Vaccinium myrtillis* L. (bilberries) have already been investigated by Willstätter and Zollinger (*Annalen*, 1915, **408**, 84; 1916, **412**, 195) and Karrer and Widmer (*Helv. Chim. Acta*, 1927, **10**, 5), the separation and identification of the individual pigments in the complex mixture remains to be accomplished. The former authors considered that myrtillin was essentially a galactoside, whereas the later workers found a mixture of glucosides and galactosides. Karrer and Widmer also established the presence of one or more malvidin derivatives and by fractionation of the picrate showed that a methoxyl-free pigment could be isolated. However, the homogeneity of even this fraction was not certain, although it appeared to be largely galactosidic.

Our own examination of the natural pigment is not yet complete, but it has indicated that in addition to malvidin and delphinidin, a petunidin glycoside is present. Focusing attention first on the malvidin derivatives, we have synthesised *malvidin chloride* 3-*galactoside* (I) in order to compare and contrast it with the 3-glucoside (oenin) and, although we are not yet able to use our results to throw fresh light on the problem of the bilberry colouring matters, there has been an interesting outcome in an unsuspected quarter.

The synthesis followed the lines of that of oenin (Levy, Posternack, and Robinson, J., 1931, 2701), but was more troublesome at every stage owing to the inferior power of crystallisation and the increased tendency to hydrolysis of the galactoside. Ultimately a pure specimen was secured and this exhibited, as was expected, very great similarity with oenin in colour reactions, but a difference in the distribution numbers was noted of such magnitude as to render identification by this means a feasible proposition.

(I.)





(II.)

As in the case of oenin, the distribution varied with the concentration and, over the range examined, the relation between the logarithms of the concentrations in the aqueous and the amyl-alcoholic layer was linear with a slope of 2. The graphs for the glucoside and the galactoside on this basis lie parallel and at a more than sufficient distance for practical purposes. We accordingly thought it desirable to examine the distribution numbers of cyclamin chloride (Karrer and Widmer, *Helv. Chim. Acta*, 1927, **10**, 758) and primulin chloride (Scott-Moncrieff, *Biochem. J.*, 1930, **24**, 767), which are known to be malvidin 3-glycosides. A specimen of cyclamin picrate from *Cyclamen persicum* was kindly sent to us by Professor P. Karrer, and its distribution numbers at three different concentrations agreed closely with those of oenin. There can therefore be no doubt that cyclamin and oenin are identical.

A pure specimen of primulin * from *Primula polyanthus* was not available, but Miss R. Scott-Moncrieff has also isolated the anthocyanin of *P. sinensis*, and several specimens of this have been examined. The results indicated clearly that this pigment is identical with our malvidin 3-galactoside, although it appears that the colouring matters of different varieties are not quite identical.

This was confirmed by a study of the orcinol–sulphuric acid reaction (compare Tillmans

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* We understand that Miss Scott-Moncrieff, if she confirms our findings, will propose the name *primulin* for the anthocyanin of *P. sinensis*: this can cause no confusion, because the anthocyanin of *P. polyanthus* will be either primulin (galactoside) or oenin and in the latter case the use of a special name becomes unnecessary.

and Phillippi, *Biochem. Z.*, 1929, **215**, 36; Sörensen and Hangaard, *Compt. rend. Trav. Lab. Carlsberg*, **19**, No. 12), which serves admirably to distinguish very small quantities of glucose from equal amounts of galactose. Equivalent solutions of oenin and of malvidin 3-galactoside were hydrolysed by means of aqueous sulphuric acid and after removal of the anthocyanidin the residual sugar solutions were used for the development of the orcinol colour reaction. Again the results showed that the *P. sinensis* anthocyanin is the galactoside and not the glucoside.

We have also obtained solutions of *malvidin chloride* 3-*xyloside* (II), but, as in the case of cyanidin chloride 3-xyloside (Part XVIII; this vol., p. 806), hydrolysis occurs very readily and several specimens were completely destroyed before this was suspected. The distribution number was determined to our satisfaction.

EXPERIMENTAL.

O-*Trimethylgallic Acid.*—An improved yield was obtained under the following conditions (compare Perkin and Weizmann, J., 1906, **89**, 1655). A mixture of gallic acid (150 g.), water (375 c.c.), and methyl sulphate (339 c.c.) was cooled in ice-water and vigorously stirred, air being displaced by hydrogen. Aqueous sodium hydroxide (1905 c.c. of 15%) was introduced at such a rate that no large rise of temperature occurred; thereafter, stirring was continued for 1 hour, and the liquid filtered. The residual methyl trimethylgallate was separately hydrolysed by means of hot aqueous sodium hydroxide, and the clear solution added to the main bulk. The trimethylgallic acid was isolated after acidification of the solution and crystallised from 5% aqueous acetic acid (charcoal) (m. p. 164—165°; yield, 145 g., or 78%).

ω-O-*Diacetyl*-β-*galactosidoxy*-4-*acetoxy*-3 : 5-*dimethoxyacetophenone*,
$(AcO)_2(HO)_2C_6H_7O{\cdot}O{\cdot}CH_2{\cdot}CO{\cdot}C_6H_2(OMe)_2{\cdot}OAc$.
—Dry silver oxide (18 g.) was added to a solution of ω-hydroxy-4-acetoxy-3 : 5-dimethoxyacetophenone (6 g.) (Levy, Posternack, and Robinson, *loc. cit.*) and *O*-tetra-acetyl-α-galactosidyl bromide (13·4 g.) in dry benzene (36 c.c.) at 40°. The mixture was agitated and the temperature rose to 70—80°; shaking was continued for ½ hour and the mixture was again gently heated and filtered. Light petroleum (240 c.c.) was added to the cooled filtrate, precipitating a brownish-yellow syrup, which was washed and triturated with hot and then with cold water. The product was dissolved in hot methyl alcohol (10 c.c.) (charcoal) and precipitated from the filtrate by water; repetition of this process gave a clean white solid, which, however, could not be crystallised (yield, 6·3 g. or 45%) (Found : C, 52·5; H, 5·5; MeO, 12·7. $C_{22}H_{28}O_{13}$ requires C, 52·8; H, 5·6; MeO, 12·4%). The substance is evidently only a triacetate, but an alternative to the formula represented above is that containing three acetoxy-groups in the galactose residue and a phenolic hydroxyl.

3-β-*Galactosidylmalvidin Salts* (I).—Dry hydrogen chloride was passed for 2 hours into a solution of 4-hydroxy-ω-galactosidoxy-3 : 5-dimethoxyacetophenone triacetate (2·1 g.) and 2-*O*-benzoylphloroglucinaldehyde (1·8 g.) in dry ethyl acetate (28 c.c.), and the mixture kept for 72 hours. The red liquid was filtered, and the flavylium *salt* precipitated by the addition of ether (180 c.c.) in minute prisms (air-dried, 2·3 g. or 78%) (Found : C, 54·3; H, 4·8; Cl, 7·8. $C_{36}H_{35}O_{16}Cl,HCl$ requires C, 54·3; H, 4·5; Cl, 8·9%). This result shows that the salt is essentially a dihydrochloride; replacement of a part of the HCl by H_2O is also indicated. This benzoylated flavylium salt (0·67 g.) was dissolved in barium hydroxide solution (40·2 c.c. of 0·256*N*) under hydrogen, and the solution kept for 12 hours. It was then neutralised with sulphuric acid (4·9 c.c. of 2·10*N*) and centrifuged, and the supernatant liquid filtered and added to an equal volume of saturated aqueous picric acid. The anthocyanin *picrate* thus precipitated was crystallised by solution in the minimum quantity of warm ethyl alcohol and addition of hot saturated aqueous picric acid, forming bunches of fine orange-red needles with a green reflex (Found: C, 46·1; H, 4·1; N, 6·1. $C_{29}H_{27}O_{19}N_3,2H_2O$ requires C, 46·0; H, 4·1; N, 5·5%). This derivative was moderately readily soluble in 0·5% hydrochloric acid and was not extracted from the solution by means of saturated ethereal picric acid; it was therefore free from malvidin.

The crystallised picrate was dissolved in cold 4% methyl-alcoholic hydrogen chloride, and the chloride precipitated by means of ether, washed, and dried. It was dissolved in cold 1% methyl-alcoholic hydrogen chloride, and the acid concentration increased to 4%; on keeping, *malvidin chloride* 3-*galactoside* crystallised in fine needles and pointed prisms exhibiting a bronze metallic glance [Found : C, 49·4, 49·2; H, 5·1, 5·1; Cl, 6·7, (7·7); MeO, 11·8, 11·6. $C_{23}H_{25}O_{12}Cl,1{\cdot}5H_2O$ requires C, 49·7; H, 5·0; Cl, 6·4; MeO, 11·2%]. The crystals resemble those of oenin chloride and are bluish-violet by transmitted light. The specimen was entirely free from anthocyanidin as shown by the ether–picric acid test.

Addition of lead acetate to a solution in 0·5% hydrochloric acid gives at first a violet-blue solution and a dark blue precipitate, but, on keeping, the solution becomes colourless as the whole of the pigment is precipitated.

The colour reactions of this salt are identical with those of oenin chloride and a direct comparison was carried out with buffered solutions of graded p_H. No differences could be detected over the whole range and these results have already been recorded (Part VIII, *loc. cit.*, p. 2714).

The distribution numbers (*iso*amyl alcohol and 0·5% hydrochloric acid) were determined as described by Levy and Robinson (J., 1931, 2720).

Mg.	G.-mols. $\times 10^6$.	Distrib. number.	Log C_W.	Log C_{AA}.	$K = 100C_{AA}^2/C_W$.
7·399	13·10	9·7	1·0730	0·1023	13·5
5·919	10·48	10·9	0·9703	0·0570	13·9
4·439	7·86	12·3	0·8382	$\bar{1}$·9848	13·5
2·960	5·24	14·4	0·6522	$\bar{1}$·8771	12·7
1·480	2·62	18·6	0·3284	$\bar{1}$·6870	11·1

The approximately linear relation of log C_W with log C_{AA} is shown in Fig. 1.

Absorption coefficients were determined with a solution ($0{\cdot}18N \times 10^{-4}$) in 0·1% methyl-alcoholic hydrogen chloride and a cell of 20 mm. As with oenin, the maximum occurs at λ 5400 μμ (Fig. 2).

FIG. 1.

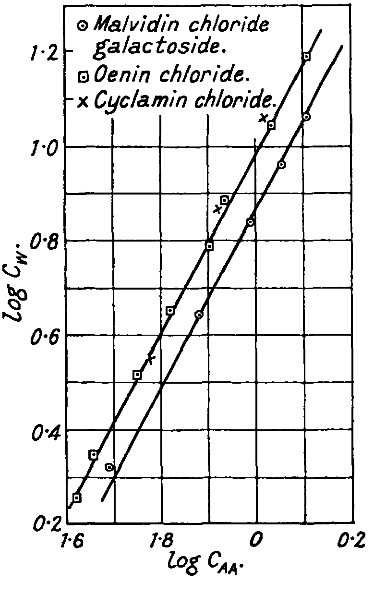
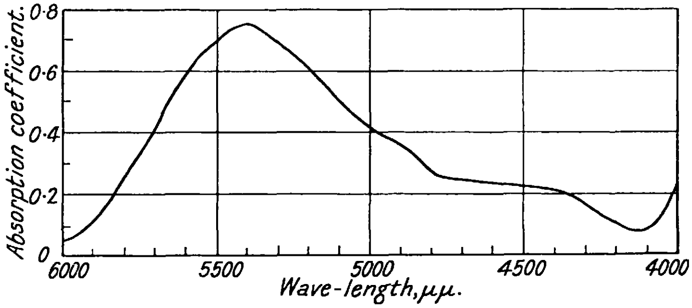


FIG. 2.



Examination of Cyclamin.—The picrate (25·1 mg.) supplied by Professor Karrer was dissolved by gentle heating with 0·5% hydrochloric acid (50 c.c.) and *iso*amyl alcohol (10 c.c.); the solution was then mixed with benzene (50 c.c.) and separated after vigorous shaking. The aqueous solution was extracted seven times with benzene (total, 300 c.c.); the last two extracts were free from picric acid. The solution was washed twice with *iso*amyl alcohol (10 c.c.), filtered, and colorimetrically estimated, oenin chloride being used as a standard. The distribution ratios were then determined for three concentrations :—

Mg.	G.-mols. $\times 10^6$.	Distrib. number.	Log C_W.	Log C_{AA}.	K.
7·333	12·99	8·09	1·0773	0·0168	9·0
4·899	8·66	9·42	0·8743	$\bar{1}$·9116	8·5
2·430	4·18	14·3	0·5486	$\bar{1}$·7750	10·0

The relation between log C_W and log C_{AA} is shown in Fig. 1, and for comparison the values for oenin chloride are reproduced.

There can be no doubt that cyclamin is malvidin 3-β-glucoside.

Examination of the Anthocyanin of Primula Sinensis.—The first two specimens submitted by Miss R. Scott-Moncrieff gave distribution numbers intermediate between those required for the glucoside and the galactoside of malvidin but with a closer approach to the latter (Fig. 3). Owing to some doubt about the composition of the substance the results are calculated for $2H_2O$, $3H_2O$, and $4H_2O$. Later a purer specimen was acquired and this gave the following results :—

Mg.	G.-mols. × 10^6.	Distrib. number.	Log C_W.	Log C_{AA}.	K.
9·611	15·31	8·76	1·1452	0·1275	12·87
7·074	11·27	10·31	1·0047	0·0655	13·38
4·806	7·66	12·67	0·8254	$\bar{1}$·9868	14·06
2·403	3·83	17·08	0·5024	$\bar{1}$·8158	13·47
2·358	3·76	16·39	0·4969	$\bar{1}$·7898	12·10
1·179	1·88	19·94	0·1790	$\bar{1}$·5738	9·30

Fig. 4 shows the graph log C_W–log C_{AA} for malvidin chloride 3-galactoside with the points derived from the above data for comparison. These results were confirmed by the use of the orcinol reaction in the following manner.

FIG. 3.

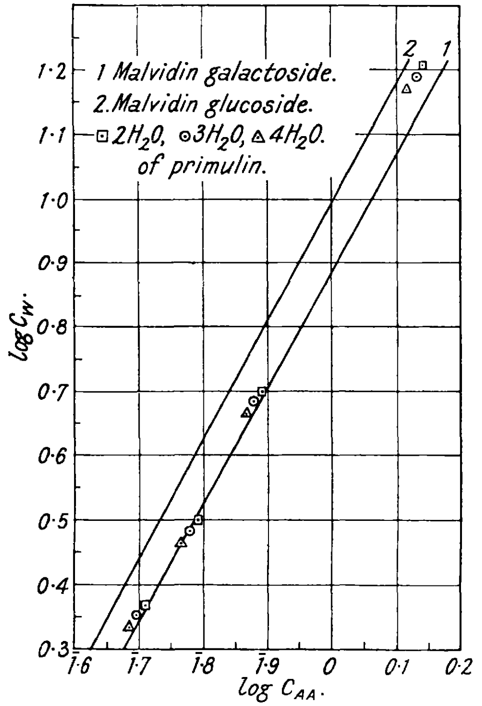
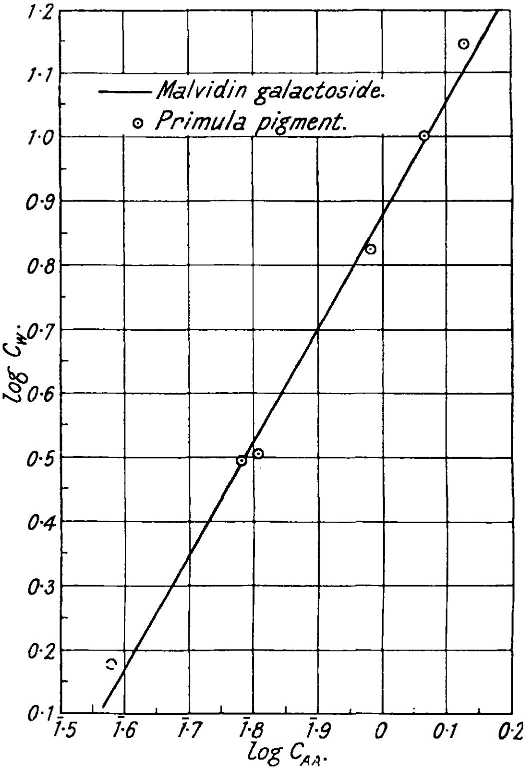


FIG. 4.



Distribution ratios of malvidin galactoside and of pigment of Primula sinensis.

Solutions of oenin chloride, malvidin chloride galactoside, and the pigment from rose-coloured flowers of *Primula sinensis* were prepared and made up to the same concentration (about 5·0 mg. in 100 c.c.) by colorimetric comparison; the anthocyanins were then hydrolysed by heating on the steam-bath for 1 hour after the addition of one-third of a volume of concentrated sulphuric acid. The malvidin was completely removed from the cooled solutions by means of amyl alcohol and the aqueous solutions were then washed with pure benzene and with light petroleum previously distilled over caustic potash. Any slight differences in total volume were then adjusted. A 2% solution of orcinol in sulphuric acid (1 vol. of concentrated acid mixed with 3 vols. of water) and a mixture of 3 vols. of concentrated sulphuric acid and 1 vol. of water were used as stock reagents. The sugar solution from the anthocyanin (1 c.c.) was mixed with the orcinol solution (2 c.c.) and the 3 : 1 sulphuric acid (15 c.c.); this was done with each of the three specimens and the flasks were then placed together in a water-bath at 80—90° and heated for the same length of time. A yellowish-orange coloration resulted and the intensities were compared colorimetrically, the malvidin galactoside being used as standard in each case.

	Time (mins.).	Galacto-side.	Oenin.	Primulin.		Time (mins.).	Galacto-side.	Oenin.	Primulin.
(1)	5	1	0·63	1·05	(2)	5	1	0·79	1·02
	10	1	0·61	0·89		10	1	0·83	1·02
	15	1	0·76	1·4		15	1	0·77	1·03
	30	1	0·82	1·2		30	1	0·74	0·90

In the first series the *P. sinensis* pigment had been standing for some time and the slightly higher results are probably due to formation of colourless or pale-coloured galactosides by decomposition. On colorimetric adjustment of the concentration any such substances would remain as additional sources of galactose. The second series was carried out with the purest specimens available and fresh solutions.

Under the conditions described above, galactose was found to give a much deeper colour than glucose. The amount of hexose used in each of the tests was less than 0·01 mg.

ω-O-Triacetyl-β-xylosidoxy-4-acetoxy-3 : 5-dimethoxyacetophenone.—Active silver oxide (4·4 g.) (compare Helferich and Klein, *Annalen*, 1926, **450**, 256) was added to a solution of ω-hydroxy-4-acetoxy-3 : 5-dimethoxyacetophenone (4·8 g.) and triacetylxylosidyl bromide (7·2 g.) in dry benzene (29 c.c.) at about 40°; the temperature rose to 80° and after ½ hour the mixture was gently heated and filtered. The syrupy xyloside was precipitated by means of light petroleum and triturated with hot and with cold water; it was then precipitated from methyl-alcoholic solution (charcoal) by means of water and on repetition of this process the derivative crystallised (yield, 3 g. or 47%), m.p. 70—73° after drying in a vacuum over phosphoric oxide (Found : C, 53·7; H, 5·5. $C_{23}H_{28}O_{13}$ requires C, 53·9; H, 5·5%).

Malvidin Chloride 3-Xyloside (II).—A solution of triacetylxylosidoxyacetoxydimethoxyacetophenone (3·5 g.) and 2-*O*-benzoylphloroglucinaldehyde (3·5 g.) in dry ethyl acetate (50 c.c.) was cooled to 0°, saturated with hydrogen chloride, and kept for 3 days in the ice-chest. A red crystalline deposit (A) was collected; the flavylium *salt* was precipitated from the filtrate by means of ether, washed, and dried (yield, 0·8 g.) (Found : C, 57·4; H, 4·6; Cl, 5·0. $C_{37}H_{35}O_{16}Cl$ requires C, 57·6; H, 4·5; Cl, 4·6%). This material appeared to be the xyloside, but (A) is already hydrolysed (Found: C, 60·0; H, 4·1; Cl, 7·2. $C_{26}H_{21}O_9$ requires C, 60·9; H, 4·1; Cl, 7·1%), and in fact it is probably *5-O-benzoyl-4′-O-acetylmalvidin chloride.* The substance was composed of well-defined orange-red needles, differing from benzoylmalvidin chloride; it gave a red solution in alcohol and a blue solution in aqueous sodium carbonate. On hydrolysis by means of methyl-alcoholic barium hydroxide and re-formation of the flavylium salt, an exceptionally pure specimen of malvidin was obtained.

The acetylated xyloside precipitated by ether lost its sugar on hydrolysis under most conditions and after several unsuccessful attempts the following procedure was adopted. The crude product (1·9 g.) was dissolved in the minimum quantity of 0·5% methyl-alcoholic hydrogen chloride and, after exclusion of air with hydrogen, an excess of dilute methyl-alcoholic baryta was added. The barium was exactly precipitated by methyl-alcoholic sulphuric acid after 6 hours, and the anthocyanin precipitated from the filtered solution by means of ether. This material was not free from barium salts; it was dissolved in alcohol, and the filtered solution mixed with saturated aqueous picric acid. Only a small proportion of the picrate separated and the solution was concentrated in a vacuum at room temperature. The picrate was collected and precipitated again from an aqueous-alcoholic picric acid solution. (It was completely hydrolysed by solution in cold 4% methyl-alcoholic hydrogen chloride and precipitation of the chloride with ether.)

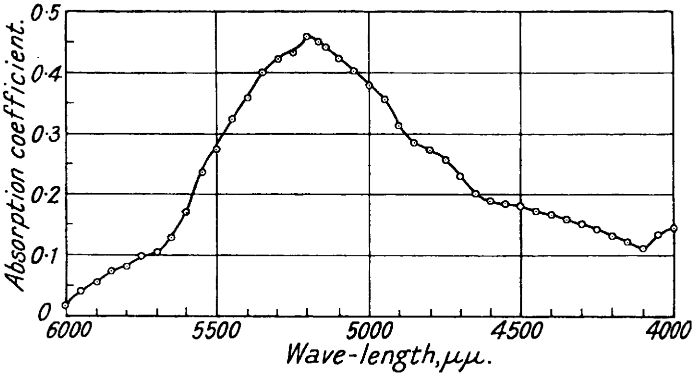
The picrate was shaken with 0·5% hydrochloric acid, and the resulting solution repeatedly washed with ethereal picric acid in order to remove all traces of malvidin. Picric acid was then removed by means of ether, toluene, and ether, and the ether was removed in a stream of air. This solution was estimated by colorimetric comparison with oenin chloride, allowance being made for the small difference in molecular weight.

The distribution numbers with *iso*amyl alcohol were found to be independent of the concentration : 8·09 mg. in 50 c.c. of the mixed solvents, D.N. 45·3; 2·70, 45·3; 1·98, 45·7; 1·76, 43·9; very dilute, 46·0.

The absorption was measured in an aqueous solution of 1 mg. in 100 c.c. (Fig. 5). The colour in water is not so bright as in alcohol.

The colour reactions in solutions of graded p_H were examined, 1·98 mg. in 25 c.c. being used. As the acid concentration was higher than normal, a corresponding oenin solution was used for comparison (2·16 mg. in 25 c.c. of 0·5% acid), and for the same reason the results are somewhat different from those previously recorded. The oenin observations are in parentheses. (1) Rose (rose); (3) rose (rose); (5) slightly paler rose (slightly paler rose); (7) like (5) (rather bluer rose); (9) bluish-rose (like 7); (11) reddish-violet (rose with blue shade); (13) slaty-blue (pale red-violet); (15) violet-blue (like 13); (16) blue (pale blue, fading); (17) blue (like 16).

FIG. 5.



Absorption spectrum of malvidin chloride xyloside.

After 1½ hours : (1) to (7) graduated series of rose, decreasing in intensity in both cases; (9) very pale pink (almost colourless); (11) like (9) (colourless); (13) pale violet (nearly colourless); (15) violet-blue (pale reddish-violet); (16) blue (blue); (17) yellow (yellow).

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